

**TRANSPORT STUDIES ON *GOMPHOCARPUS PHYSOCARPUS*:
OBSERVATIONS ON THE FEEDING HABIT OF *APHIS NERII*. B. de F.***

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ABSTRACT

Scanning electron and light microscope studies carried out on the feeding habit of the aphid, *Aphis nerii*, on *Gomphocarpus physocarpus*, underscore the remarkable adaptation of the labium and of the stylet group for puncturing and penetrating plant tissues. Penetration of the stylet pairs is intercellular in the cortex and mainly intracellular in the ray parenchyma. The aphid feeds in sieve elements and, despite the greater distance of traverse, preferentially in those of the internal phloem bundles and should, therefore, be potentially useful in transport studies of dissolved organic substances.

UITTREKSEL

**TRANSLOKASIE-STUDIES OP *GOMPHOCARPUS PHYSOCARPUS*: WAAR-
NEMINGS OP DIE VOEDINGSGEWOONTE VAN *APHIS NERII*. B. de F.**

Studies wat met behulp van skanderende elektron- en ligmikroskopie op die voedingsgewoontes van die plantluis *Aphis nerii* op *Gomphocarpus physocarpus* onderneem is, bevestig die besondere aanpassing van die stiletgroep en die labium van die insek ten opsigte van die binnedringing van plantweefsel. Binnedringing van die stiletpare deur die korteks is intersellulêr maar in die straalparenchium hoofsaaklik intrasellulêr. Dié plantluis voed in die sifvatelemente en by voorkeur in dié van die interne floeëmbondels, ten spyte van die groter afstand van binnedringing as gevolg daarvan. *Aphis nerii* is dus potensieel geskik vir gebruik in translokasiestudies van opgeloste organiese stowwe.

INTRODUCTION

In recent years an increasing number of investigators have adopted techniques utilizing aphids and severed aphid mouthparts in their attempts to rationalize some of the many problems and major controversies associated with the translocation of dissolved organic substances in the phloem. Auclair (1963) has reviewed extensively the feeding habit and nutrition of aphids. However, relatively few considerations have been accorded the anatomical and histological aspects of stylet penetration of plant tissues or even, for that matter, the anatomy of the aphid's mouthparts.

Esau, Namba and Rasa (1961) found that about 50 per cent of the deeper punctures left at *Myzus persicae* in sugar beet leaves terminated in the phloem tissue. Zimmerman (1961), and Evert, Eschrich, Medler and Alfieri (1968)

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studied the penetration of *Tilia americana* branches by the large aphid *Longistigma caryae*. Evert *et al.* showed that this aphid could feed in phloem sieve elements more than a year old. Hoad, Hillman and Wareing (1971), working with *Tuberolachnus salignus*, found that radioactive indoleacetic acid and its metabolic products were transported in the phloem of *Salix viminalis* L.

In the study reported on here, *Gomphocarpus physocarpus* was selected as experimental material because of the presence of external as well as internal phloem in seedlings and the predominance of internal phloem bundles in adult plants, a feature characteristic of members of the Asclepiadaceae. Also, as far as we are aware, no work has yet appeared which reports specifically on the apparent preference of aphids for internal phloem. Our primary interest is in the study of the transport of plant growth and other substances and it appeared that *Aphis nerii* might be used quite effectively in our work with radioactive materials and particularly on intact plants rather than explants. However, in studies of this nature it is essential to understand the feeding habit of the vector and this paper reports on some of our observations. Other papers following shortly, will report on the physiological work.

MATERIALS AND METHODS

Colonies of *Aphis nerii* were established on the stems and leaves of nine-week-old seedlings and adult plants of *Gomphocarpus physocarpus*. The aphids were killed *in situ* on leaves and stem segments either by exposing the plant material to an atmosphere saturated with acrolein or by applying acrolein directly with a dropper pipette. Suitable lengths of plant tissue with the dead aphids in their *in situ* feeding positions, were then prepared for microscopic study by fixing in formalin-acetic acid-alcohol and embedding in paraffin wax (Sass, 1958). Sections were cut at 10 μm and stained with safranin and fast green (Johansen, 1947). With this staining combination the aphid's stylet sheaths were stained golden-brown against the usual green and red background and consequently were easily identified. The material used for scanning electron microscopy was simply mounted on specimen stubs by means of a 50 per cent mixture of Samsonite adhesive and silver conducting paint, and coated lightly with evaporated carbon. The specimens were viewed in a Hitachi SSM2 scanning electron microscope. Many aphids were excellently preserved by this procedure although some showed marked distortion. This can probably be attributed to the fact that

FIG. 1.

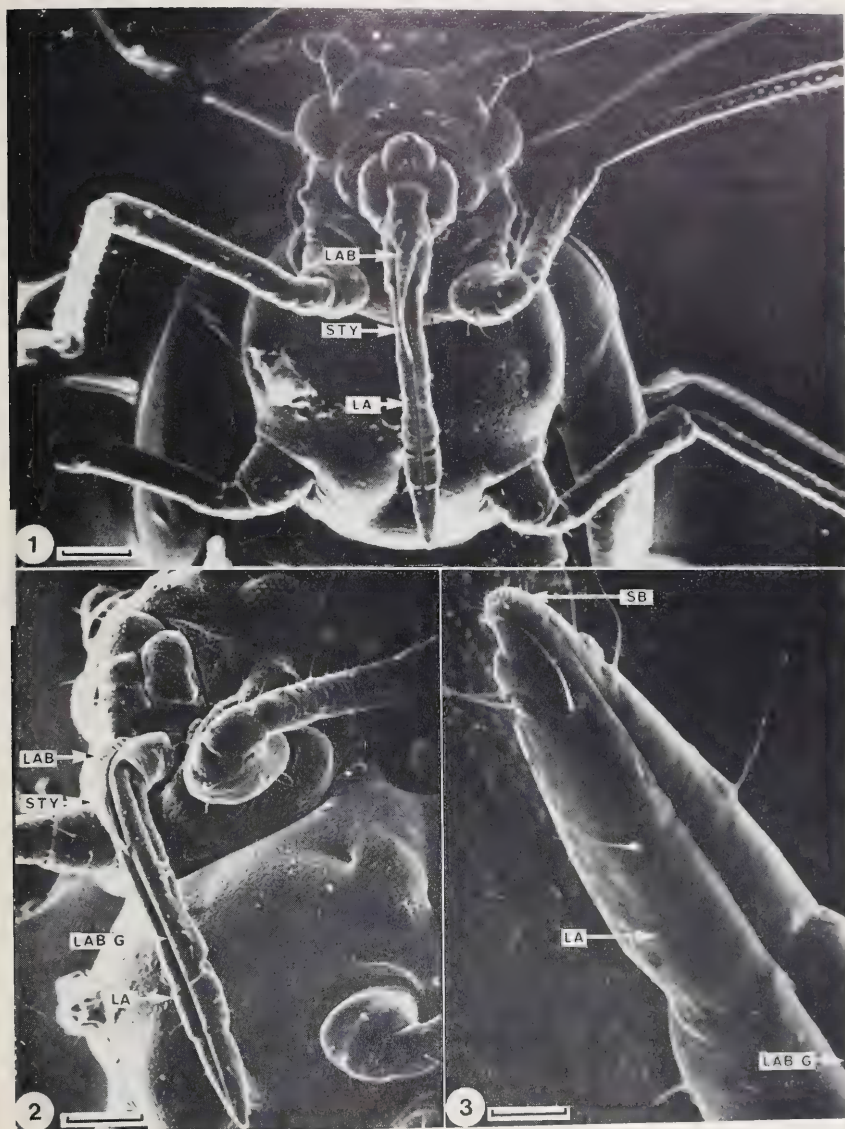
Ventral view of *A. nerii*. The labium (LA) is in the non-feeding position. Note the labrum (LAB) at the base of the labium covering the stylets (STY). Scale line = 60 μm .

FIG. 2.

Lateral view of the aphid showing labium (LA) and labial groove (LG) in which the stylets lie. Scale line = 70 μm .

FIG. 3.

Distal tip of labium, showing sensory bristles (SB). Scale line = 20 μm .



the aphids were at different stages of their life cycles, the adults being better able to withstand the vacuums to which they were subjected by virtue of their thicker, more heavily sclerotised cuticles.

RESULTS AND DISCUSSION

Aphis nerii has been found to feed almost exclusively on *Gomphocarpus physocarpus*. Figures 1–12 emphasize certain morphological features of the aphid and its mouthparts, the latter clearly well-adapted for puncturing and penetrating plant tissues. In Figs. 1 and 2 the labium (LA), which projects downward from the head, is shown in the non-feeding position, that is, with the stylets retracted; note the labial groove (LAB G) in which the two pairs of stylets lie. The proximal end of the labium contains no groove and is covered by the labrum (LAB), a structure which overlies the stylets. The distal end of the labium is surrounded by a ring of sensory bristles (Figs. 3, 4, SB) which are believed to assist the aphid in its selection of a suitable area on the plant epidermis for probing. The sensory bristles project from a muscular clamp or collar (Fig. 4) through which the stylets protrude. The labium is jointed as in most Hemiptera (Figs. 2, 3, 5 and 12) thus rendering it flexible, a function which allows it shortening during stylet penetration and probably also prevents damage to the stylets during probing operations.

The stylet anatomy of *Tuberolachnus salignus* and *Myzus persicae* was investigated by Mittler (1954) and van Hoof (1956), using transmission electron microscopy. Figures 6–9 are scanning electron micrographs of the stylets of *A. nerii* taken at high magnification ($5\,000\times$ and more) and reveal some interesting features. The mandibular stylets (Figs. 6, 8, 9, MAN STY) possess hook-like projections on their surfaces about $5.0\ \mu\text{m}$ apart (Fig. 9) on the surface. The maxillary stylets possess a series of lateral tooth-like projections (large white arrows) about $1.0\ \mu\text{m}$ apart and about 5.0 – $6.5\ \mu\text{m}$ from the apices of the maxillary stylets (Figs. 6 and 9). They are presumed to play a part in the penetration of the stylet group into the plant tissue.

A noticeable feature is the overlapping of the maxillary stylets themselves (Fig. 7). Serial transverse and longitudinal sections of paraffinembedded tissue (Figs. 10–12), provided information on the method of stylet penetration.

FIG. 4.

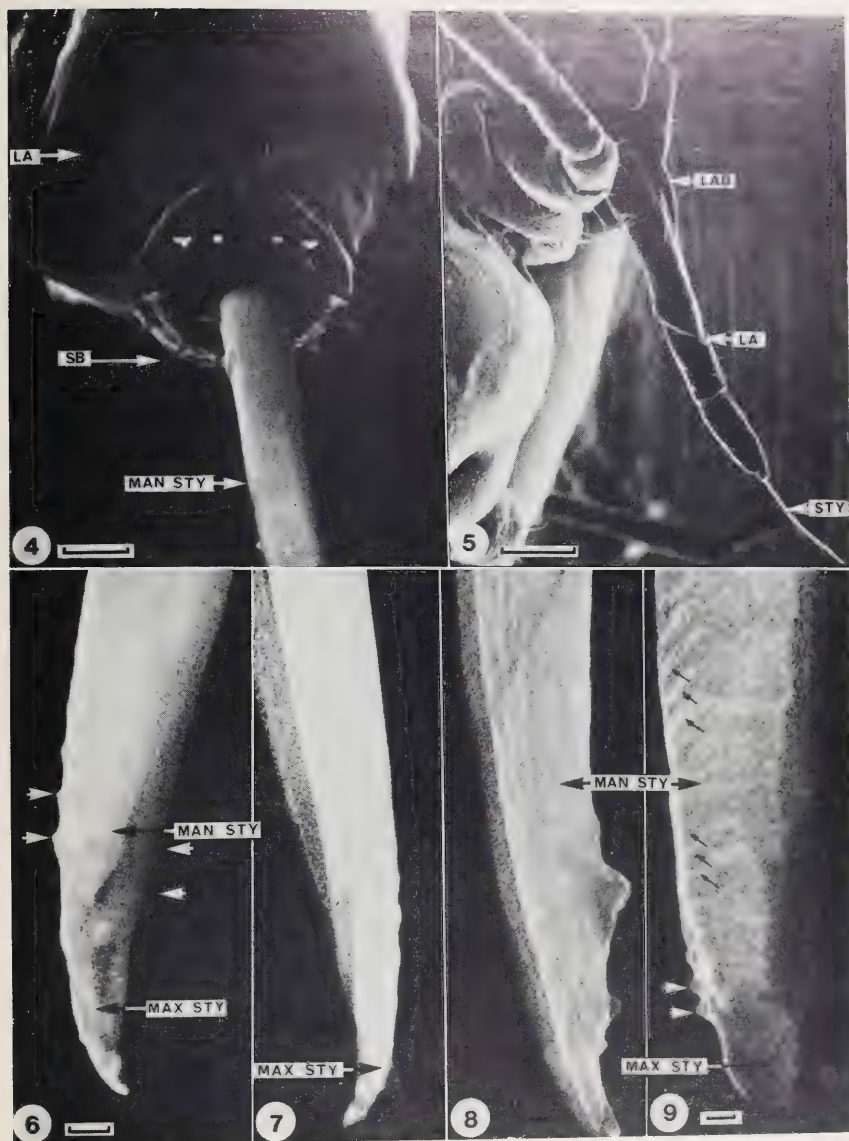
Distal end of labium showing mandibular stylets (MAN STY) protruding. Scale line = $4.5\ \mu\text{m}$.

FIG. 5.

Lateral view of *Aphis nerii* showing mouthparts. Scale line = $70\ \mu\text{m}$.

FIG. 6–9.

Aspects of the stylet tips. Scale lines represent $1.0\ \mu\text{m}$ (Figs. 6–8), and $0.5\ \mu\text{m}$ (Fig. 9). Fig. 6. Lateral view of mandibular (MAN STY) and maxillary stylets (MAX STY). Note tooth-like projections (large white arrow heads). Fig. 7. Ventral view of maxillary stylets. Figs. 8–9. Oblique views of stylet groups. Note the ridges (small arrows) on the mandibular stylets and lateral tooth-like projections (large white arrow heads).



Generally, the stylets appear to be inserted between cell walls (Fig. 13). The stylet track or sheath formed within the plant is nearly twice as wide, from 5.0 to 7.5 μm , than the diameter of the stylets (3.0—3.8 μm). This would seem to result from the action of the saliva which is injected through the maxillaries, and which probably contains enzymes involved in the dissolution of the middle lamella and cell wall substances.

Judged by the intensity of the staining reaction, it appears that less saliva is secreted during intercellular penetration of cortical tissue than is the case during penetration of vascular tissue, particularly through a field of xylem. In the latter instance the saliva sheath often appears puffy in outline (Fig. 13); presumably this is linked to the greater difficulty encountered in penetration and consequently to a more liberal release of the salivary secretions. Where a xylem vessel is punctured, or where the saliva secretion is forced into such a vessel, probably through pits, the gelled saliva obliterates a large portion of the cavity, effectively sealing the track (Fig. 13, lower centre). Upon examination of transverse sections, the distal tips of the stylet sheaths when terminating in sieve elements frequently appeared to be sealed off. Evert *et al.* (1968) interpreted this as being indicative of non-conducting phloem; in other words there is no evidence of surging, an action which would otherwise be associated with functional elements. By no means do all tracks lead to functional phloem. Esau *et al.* (1961) pointed out that the deposition of saliva in sieve elements could interfere with the feeding process by interrupting the transport of sap.

The results of an examination of 10 sections each of young and adult plants containing stylets and stylet sheaths are given in table one on page 202.

In the seedling and adult plant 60 and 80 per cent of the penetrations, respectively, terminated in the internal phloem, despite the greater distance of traverse. The number of functional sieve elements punctured are also greater in the internal phloem.

FIG. 10.

Photomicrograph of longitudinal section through adult *Gomphocarpus physocarpus* stem, showing relationship of *A. neri* to the stem during feeding. Scale line = 160 μm .

FIG. 11.

Photomicrograph of transverse section through a young 9-week *G. physocarpus* stem and the head of *A. neri* in feeding position with its stylets protruding through the labium and penetrating the epidermis. Scale line = 160 μm .

FIG. 12.

Photomicrograph of a longitudinal section through labium (LA). Two pairs of stylets—outer mandibular (MAN STY) and inner maxillary (MAX STY)—as well as labial groove (LAB G) are visible. Scale line = 40 μm .

FIG. 13.

Photomicrograph of a transverse section of *G. physocarpus* showing stylets and stylet tracks of *A. neri*. Details: PAR, parenchyma; PFI, phloem fibre initial; LAT, laticifer; EPSE, external phloem sieve element; EPCC, external phloem companion cell; STY T, stylet track; XP, xylem parenchyma; MXV, metaxylem vessel; IPSE, internal phloem sieve element; IPCC, internal phloem companion cell.



TABLE 1

Average length of stylet sheaths of *Aphis nerii* in young and adult *Gomphocarpus physocarpus* with reference to number of penetrations of functional sieve elements.

	Ave Distance in μm from Epidermis to:		
	External Phloem	Internal Phloem	Centre of Stem
9-Week-Seedling	320	439	931
No. of penetrations	4	6	
No. of functional sieve elements	4	5	
No. of branched stylet sheaths	4	2	
Adult Plant	341	594	1333
No. of penetrations	2	8	
No. of functional sieve elements	1	7	
No. of branched stylet sheaths	1	1	

The tracks or sheaths frequently appear forked in the vicinity of the vascular tissue (Fig. 13). Evert *et al.* (1968) also reported that in *Tilia americana* branching of the stylet tracks usually occurred in the region of living sieve elements. In young *G. physocarpus* seedlings branching of the stylet tracks may be influenced by the very narrow diameter of some of the sieve elements, some of which were of the order of only 10 μm . In adult *G. physocarpus* stylet tracks were found to follow a fairly straight course through the cortex and ray parenchyma of the xylem, ending usually in a bundle of internal phloem. Mittler (1957) suggested that the branching phenomenon in phloem may reflect the aphid's response to a local exhaustion of sieve-tube sap or even to a lack of sufficient pressure for the aphid to continue its feeding at a normal rate.

CONCLUSION

Observations on the mode of penetration of the stylet pairs and the termination of the stylet sheaths leave no doubt that *Aphis nerii* feeds in the sieve elements, particularly in those of the internal phloem bundles. This aphid, although small, is therefore potentially useful in transport studies of dissolved organic substances in *Gomphocarpus physocarpus*. Pending further intensive investigation we are led to surmise, at this stage, that the predominance of stylet sheaths terminating in the internal phloem is an indication of its sieve elements being the more functional in the translocation of photosynthates. Penetration of the stylets is mostly intercellular in the cortex and mainly intracellular in the ray parenchyma. Scanning electron microscopy of the labium and stylet pairs confirm their remarkable adaptation for puncturing and penetrating plant tissues.

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